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COMPLETE SPECIFICATION

Title of Invention

Novel burst-free sustained release poly-(lactide/glycolide) microspheres

Name, address and nationality of  
applicant(s) as in international  
application form

UNITED STATES GOVERNMENT REPRESENTED BY THE SECRETARY OF THE  
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NOVEL, "BURST-FREE" SUSTAINED RELEASE POLY-  
(LACTIDE/GLYCOLIDE) MICROSPHERES

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FIELD OF THE INVENTION

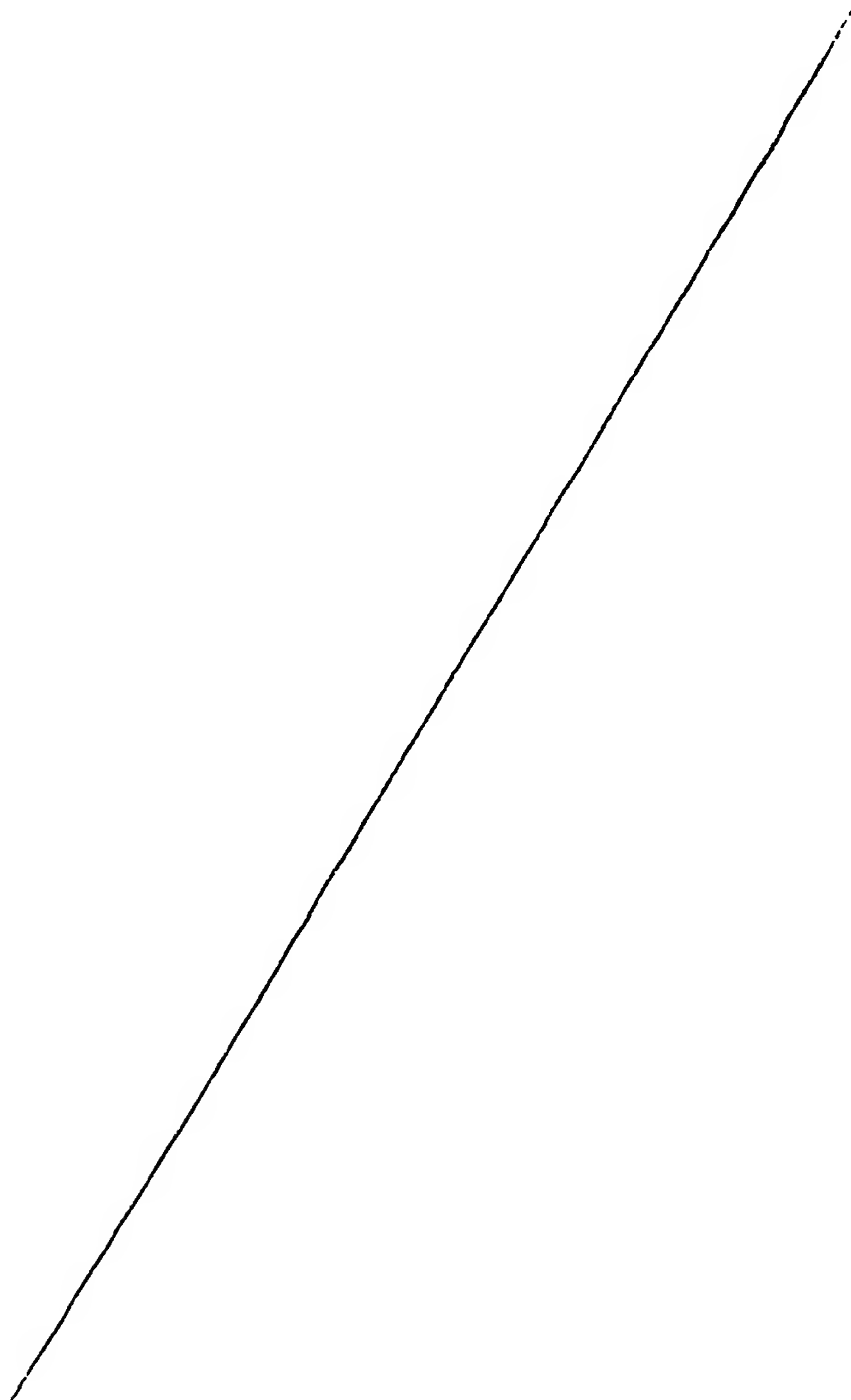
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This invention relates to providing novel biocompatible and biodegradable microspheres for burst-free programmable sustained release of biologically active agents, inclusive of polypeptides, over a period of up to 100 days in an aqueous physiological environment.

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## BACKGROUND OF THE INVENTION

Several publications and patents are available for sustained  
5 release of active agents from biodegradable polymers,  
particularly, poly(lactide/glycolides) (PLGA). Prior usages of  
PLGA for controlled release of polypeptides have involved the use  
of molar ratios of lactide/glycolide (L/G) of 75/25 to 100/0 for  
molecular weights >20,000. Further prior art preparations of PLGA  
10 utilized fillers or additives in the inner aqueous layer to  
improve the stability and encapsulation efficiency and/or to  
increase the viscosity of the aqueous layer, thereby modulating  
polymer hydrolysis and the biologically active agent or  
polypeptide release.

15 In addition, the prior art use of PLGA copolymers were end-  
capped, in that the terminal carboxyl end groups were blocked. In  
these end-capped co-polymers, the microcapsule preparations  
exhibited a low to moderate burst release of - 10-40% of the  
20 entrapped polypeptide in the first 24 hours after placement in an  
aqueous physiological environment. In part, these characteristics  
are due to the use of fillers in the inner aqueous phase.  
Further, a 1-month release of polypeptide is known with the use  
of a 75/25 co-polymer of PLGA of Mw <20,000.

25 Investigations in controlled release research has been  
proceeding especially to obtain a 1 to 2 month delivery system

for biologically active agents or polypeptides using  
poly(lactide/glycolide) polymers. However, most of these systems  
have one or more of the following problems: Poor encapsulation  
efficiency and large 'burst release' followed by an intermediate  
5 'no release' or 'lag phase' until the polymer degrades. In  
general, release from these polymers occur over a period from  
about 4 weeks to about several months. In addition, in order to  
achieve this release a 50/50 copolymer of MW > 30,000 or a 75/25  
copolymer of MW > 10,000 are employed which often results in  
10 residual polymer remaining at the site of administration long  
after the release of active core.

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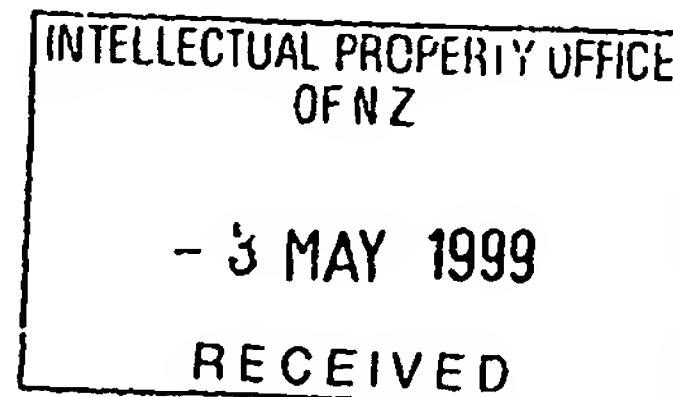
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SUMMARY OF THE INVENTION

This invention provides biocompatible and biodegradable microspheres that have been designed for novel, burst free, programmable sustained release of biologically active agents, including polypeptides over a period of up to 100 days in an aqueous physiological environment.

The use of the microspheres for human administration is claimed in New Zealand patent application No 335409 which has been divided from this application

Unlike currently available release systems, which rely on the use of fillers/additives such as gelatin, albumin, dextran, pectin, polyvinyl pyrrolidone, polyethylene glycol, sugars, etc., and are still prone to low encapsulation efficiencies and "burst effects", this invention achieves high encapsulation and "burst-free" release without the use of any additive. In this invention, burst-free, programmable sustained release is achieved through the use of a unique blend of the 'uncapped' and end-capped forms of poly(lactide/glycolide) polymer in the molecular weight range of 2,000 to 60,000 daltons.

In general, microspheres described in this invention are produced by a unique emulsification technique wherein an inner water-in-oil (w/o) emulsion is stabilized by dispersing in a solvent-saturated aqueous phase containing an emulsion stabilizer. A ternary w/o/w emulsion is then formed by emulsifying the above w/o emulsions in an external pre-cooled aqueous phase containing an o/w emulsifier. Essentially, the



inner w/o emulsion is comprised of an aqueous layer containing from - 2 to about 20% (w/w) of the active agent to be entrapped and an oil layer containing poly(lactide/glycolide) copolymer in concentrations ranging from - 5 to about-- 50% (w/w oil phase).

5 The copolymer includes molecular weight ranging from 2,000 to about 60,000 daltons, with molar composition of lactide/glycolide from 90/10 to 40/60 and a blend of its uncapped and end-capped forms in a ratio of 100/0 to 1/99. Very high encapsulation efficiencies of about 80 to 100% are achieved depending on  
10 polymer molecular weight and structural form.

Programmable release of active core over variable durations between 1-100 days is achieved by a judicious selection of process parameters such as polymer concentration, peptide concentration and the aqueous/oil phase ratio.

15 This invention is particularly suitable for high encapsulation efficiencies and burst-free, continuous programmable release of polypeptides of molecular weights ranging from 1,000 to about 250,000 daltons, and also other biologically active agents over a period of 1-100 days. A uniqueness of the  
20 invention is that when using a 100/0 blend of the uncapped and capped polymer, the final phase of active core release is concurrent with the complete solubilization of the polymer to innocuous components, such as lactic and glycolic acids. This is a significant advantage over the currently available 30 day -  
25 release systems wherein a major regulatory concern is about toxicity of residual polymer at the site of administration, long

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after release of the active core.

The microcapsules described in this invention are suitable for administration via several routes such as parenteral (intramuscular, subcutaneous), oral, topical, nasal, rectal and vaginal routes.

#### BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 shows a comparison of drug release from a conventional system versus a controlled release system. Peak and valley levels from conventional administrations are shown, in contrast to the steady therapeutic levels from the controlled release administration.

FIG. 2 shows a scanning electron micrograph of PLGA microspheres prepared by the process described in the invention using 50/50 uncapped polymer of Mw 8-12k dalton and shows superior sphere morphology, sphere integrity, and narrow size distribution.

FIG. 2a shows a scanning electron micrograph of PLGA microspheres prepared by conventional solvent evaporation method using a 50/50 12k uncapped polymer of Mw 8-12k dalton.

FIG. 3 shows cumulative Histatin release from PLGA microspheres, wherein release profiles from several batches are prepared using 50/50, uncapped polymer (of Mw 8-12k dalton) and wherein the process parameters are varied to modulate release between 1 and 35 days.

FIG. 4 shows a scanning electron micrograph of solid, smooth

spherical surfaces of PLGA microspheres prepared by the method of in the invention using 50/50, end-capped polymer (of Mw 30-40k dalton).

FIG. 5 shows cumulative Histatin release from PLGA microspheres, wherein the release profiles are from several batches prepared using 50/50, uncapped and end-capped polymer of Mw 30-40k daltons, and wherein the process parameters are varied to modulate release between 28 to 60 days.

FIG. 6 shows cumulative Histatin release from PLGA microspheres, wherein combined release profiles from several batches have been prepared using 50/50, uncapped and end-capped polymer of Mw 8-40k daltons, while varying the process parameters to modulate release between 1 and 60 days.

FIG. 7 shows a cumulative percent release of LHRH from PLGA microspheres prepared using uncapped polymer of Mw 8-12 daltons.

#### DETAILED DESCRIPTION OF THE INVENTION

This invention relates to the design of biocompatible and biodegradable microspheres for novel, programmable sustained release of biologically active agents, including polypeptides over a period of up to 100 days in an aqueous physiological environment with little or no burst release.

Unlike currently available release systems which rely on the use of fillers/additives such as gelatin, albumin, dextran, pectin, polyvinyl pyrrolidone, polyethylene glycol, sugars, etc., and are still prone to low encapsulation efficiencies and "burst effects", this invention achieves high encapsulation efficiency



and 'burst-free' release without the use of any additive. In this invention, burst-free, programmable sustained release is achieved through the use of a unique blend of the 'uncapped' and end-capped forms of poly (lactide/glycolide) polymer.

5       The 'uncapped' form refers to "poly(lactide/glycolide) with free carboxyl end groups" which renders the polymer more hydrophilic compared to the routinely used end-capped form. Currently used 'end-capped' polymer hydrates between 4-12 weeks depending on the molecular weight, resulting in an intermediate  
10       'no release' or a 'lag phase'. The uncapped polymer hydrates typically between 5 to 60 days depending on the molecular weight, thus releasing its core continuously without a lag phase. A careful blend of the two forms and appropriate molecular weights and L/G ratios, results in a continuous release between 1 to 100  
15       days. In addition, release within this time is programmable by a judicious selection of process parameters such as polymer concentration, peptide concentration and the aqueous/oil phase ratio.

20       The copolymer in this invention includes molecular weight ranging from 2,000 to 60,000 daltons, a lactide/glycolide ratio of 90/10 to 40/60 and a blend of the uncapped/capped forms in the ratio of 100/0 to 1/99. The molecular weight of the polypeptide may be in the range of 1000 to 250,000 daltons while that of  
25       other biologically active agents may range from 100 to 100,000 daltons.

Microcapsules described in this invention are prepared by a

unique aqueous emulsification technique which has been developed for use with the uncapped polymer to provide superior sphere morphology, sphere integrity and narrow size distribution. This is accomplished by first preparing an inner water-in-oil (w/o) by  
5 mixing the solutions of polymer in an organic solvent such as methylene chloride and the biologically active agent in water. This is followed by stabilization of the w/o emulsion in a solvent-saturated aqueous solution containing an o/w emulsifier such as polyvinyl alcohol. A ternary emulsion is then formed by  
10 emulsifying the w/o emulsion in an external aqueous phase containing the same emulsifier as above at concentrations ranging from 0.25 - 1% w/v. Microcapsules are hardened upon solvent removal by evaporation, rinsed to remove residual emulsifier and lyophilized. Low temperature is used both at the time of primary  
15 emulsification (w/o emulsion formation) and during the formation of the final w/o/w emulsion to achieve stable emulsion and superior sphere characteristics.

In the context of the invention, a biologically active agent is any water-soluble hormone drugs, antibiotics, antitumor  
20 agents, antiinflammatory agents, antipyretics, analgesics, antitussives, expectorants, sedatives, muscle relaxants, antiepileptics, antiulcer agents, antidepressants, antiallergic drugs, cardiotonics, antiarrhythmic drugs, vasodilators, antihypertensives, diuretics, anticoagulants, antinarcotics, etc.

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More precisely, applicants have discovered a pharmaceutical composition and process with the following itemized features:

1. A controlled release microcapsule pharmaceutical formulation for burst-free, sustained, programmable release of a biologically active agent over a duration from 1-100 days, comprising an active agent and a blend of uncapped and end-capped biodegradable poly(lactide/glycolide).

2. The pharmaceutical formulation of item 1, wherein the biodegradable poly(lactide/glycolide) is a blend of uncapped and capped forms, in ratios ranging from 100/0 to 1/99.

3. The microcapsules of items 1 or 2 wherein the copolymer (lactide to glycolide L/G) ratio for uncapped and endcapped polymer is 52/48 to 48/52.

4. The microcapsules of items 1 or 2 wherein the copolymer L/G ratio for uncapped and end-capped polymer is 90/10 to 40/60.

5. The microcapsules of item 3 wherein the molecular weight of the copolymer is between 2,000-60,000 daltons

6. The microcapsules of item 1 wherein the biologically active agent is a peptide or polypeptide

7. The microcapsules of item 6, wherein said polypeptide is histatin consisting of 12 amino acids and having a molecular weight of 1563.

8. The microcapsules of item 7 characterized by the capacity to completely release histatin in

an aqueous physiological environment from 1-35 days with a 100/0 blend of uncapped and end-capped poly(lactide/glycolide) having a L/G ratio of 48/52 to 52/48, and a molecular weight <15,000.

9 The microcapsules of item 7 characterized by the capacity to completely release histatin in

an aqueous physiological environment from 18-40 days with a 100/0 blend of uncapped and end-capped poly(lactide/glycolide) having a L/G ratio of 48/52 to 52/48 and a molecular weight range of 28,000-40,000.

10 The microcapsules of item 7 characterized by the capacity to release up to 90% of the histatin in an aqueous physiological environment from 28-70 days with a 100/0 blend of uncapped and end-capped poly(lactide/glycolide) having a L/G ratio of 48/52 to 52/48 and a molecular weight range of 10,000-40,000 daltons

11 The microcapsules of item 7 characterized by the capacity to release up to 80% of histatin in an aqueous physiological environment from 56-100 days with a 100/0 blend of uncapped and end-capped poly(lactide/glycolide) having a L/G ratio of 75/25 and a molecular weight of < 15,000 daltons

12 The microcapsules of item 6 wherein the polypeptide is histatin having analogues of histatin with chain lengths of from 11-24 amino acids of molecular weights from 1,500-3,000 daltons and characterized by the following structures

1. D S H A K R H H G Y K R K F H E K H H S H R G Y

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2. K R H H G Y K R K F H E K H H S H R G Y R  
 3. K R H H G Y K R K F H E K H H S H R  
 4. R K F H E K H H S H R G Y R  
 5. A K R H H G Y K R K F H  
 5 6. \*A K R H H G Y K R K F H  
 7. K R H H G Y K R K F

\* D-amino acid

13 The microcapsules of item 6 wherein  
 the biologically active agent is a polypeptide Leutinizing  
 10 hormone releasing hormone (LHRH) that is a decapeptide of  
 molecular weight 1182 in its acetate form, and having the  
 structure:

p- E H W S Y G L R P G

15 14 The microcapsules of item 6 having a molecular weight of from  
 1,000 to 250,000 daltons

15 The microcapsules of items 6 or 7 or 8 or 9 or 10 or 11 or 12  
 or 13 or 14 wherein release profiles of variable rates and durations are achieved by  
 20 blending uncapped and capped biodegradable poly(lactide/glycolide) polymer in the  
 microspheres as a cocktail in variable amounts

16. The microcapsules of items 6 or 7 or 8 or 9 or 10 or 11  
 or 12 or 13 or 14 wherein release of profiles of variable rates  
 and duration are achieved by blending uncapped and capped polymer  
 25 in different ratios within the same microspheres

17 The microcapsules of item 6 wherein the polypeptide

is any of the vaccine agents against enterotoxigenic E. coli (ETEC) such as CFA/I, CFA/II, CS1, CS3, CS6 and CS17 and other ETEC-related enterotoxins.

5           18. The microcapsules of items 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 wherein the entrapped polypeptide consists of peptide antigens of molecular weight range of about 800-5000 daltons for immunization against enterotoxigenic E. coli (ETEC).

10           19. The microcapsules of items 1 or 2 or 3 or 4 or 5 wherein said biologically active agents are selected from the group consisting of water-soluble hormone drugs, antibiotics, antitumor agents, anti inflammatory agents, antipyretics, analgesics, antitussives, expectorants, sedatives, muscle relaxants,  
15           antiepileptics, antiulcer agents, antidepressants, antiallergic drugs, cardiotonics, antiarrhythmic drugs, vasodilators, antihypertensives, diuretics, anticoagulants, and antinarcotics, in the molecular weight range of 100-100,000 daltons.

20           20. The microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 wherein said biodegradable poly(lactide/glycolide) is in an oil phase, and is present in about 1-50% (w/v).

25           21. The microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 wherein concentration of the active agent is in the range of 0.1 to about 60% (w/v).

22. The microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or

8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 wherein a ratio of the inner aqueous to oil phases is about 1/4 to 1/40(v/v).

23. A process for preparing controlled release microcapsule formulations characterized by burst-free, sustained, programmable release of biologically active agents comprising: Dissolving biodegradable poly (lactide/glycolide), in uncapped form in methylene chloride, and dissolving a biologically active agent or active core in water; adding the aqueous layer to the polymer solution and emulsifying to provide an inner water-in-oil (w/o) emulsion; stabilizing the w/o emulsion in a solvent-saturated aqueous phase containing a oil-in-water (o/w) emulsifier; adding said w/o emulsion to an external aqueous layer containing oil-in-water emulsifier to form a ternary emulsion; and stirring the resulting water-in-oil-in-water (w/o/w) emulsion for sufficient time to remove said solvent, and rinsing hardened microcapsules with water and lyophilizing said hardened microcapsules.

24. A process for preparing controlled release microcapsule formulations characterized by burst-free, sustained, programmable release of biologically active agents comprising:

dissolving biodegradable poly(lactide/glycolide) in end-capped form in methylene chloride, and dissolving a biologically active agent or active core in water; adding the aqueous layer to the polymer solution and emulsifying to provide an inner water-in-oil emulsion; stabilizing the w/o emulsion in a solvent-saturated aqueous phase containing a oil-in-water (o/w)



emulsifier; adding said w/o emulsion to an external aqueous layer containing oil-in-water emulsifier to form a ternary emulsion; and stirring a resulting water-in-oil-water (w/o/w) emulsion for sufficient time to remove said solvent; and rinsing hardened  
5 microcapsules with water; and lyophilizing said hardened microcapsules.

25. The process of items 23 or 24 wherein a solvent-saturated external aqueous phase is added to emulsify the inner w/o emulsion prior to addition of the external aqueous layer, to  
10 provide microcapsules of narrow size distribution range between 0.05-500 $\mu$ m.

26. The process of items 23 or 24, wherein a low temperature of about 0-4°C is provided during preparation of the inner w/o emulsion, and a low temperature of about 4-20°C is provided  
15 during preparation of the w/o/w emulsion to provide a stable emulsion and high encapsulation efficiency.

27. The process of items wherein a 100/0 blend of uncapped and end-capped polymer is used to provide release of the active core in a continuous and sustained manner without a lag phase.

20 28. The microcapsules of items 6, wherein, when the entrapped polypeptide is active at a low pH, such as LHRH, adrenocorticotrophic hormone, epidermal growth factor, calcitonin released polypeptide is bioactive.

25 29. The microcapsules of items 6 or 7 or 8 or 9 or 10 or 11, wherein, when entrapped peptide such as histatin is inactive at a low pH, a pH-stabilizing agent of inorganic salts are added to



the inner aqueous phase to maintain biological activity of the released peptide.

30. The microcapsules of items 6 or 7 or 8 or 9 or 10 or 11 wherein, when entrapped polypeptide such as histatin is inactive at a low pH, a non-ionic surfactant such as polyoxyethylene sorbitan fatty acid esters (Tween 80, Tween 60 and Tween 20) and polyoxyethylene - polyoxypropylene block copolymers (Pluronic) is added to the inner aqueous phase to maintain biological activity of the released polypeptide.

31. The microcapsules of items 29, wherein placebo spheres loaded with the pH-stabilizing agents are coadministered with polypeptide-loaded spheres to maintain the solution pH around the microcapsules and preserve the biological activity of the released peptide in instances where the addition of pH-stabilizing agents in the inner aqueous phase is undesirable for the successful encapsulation of the acid pH sensitive polypeptide.

32. The microcapsules of item 30 wherein placebo spheres loaded with non-ionic surfactant are coadministered with polypeptide-loaded spheres to maintain biological activity of the released peptide where the addition of non-ionic surfactants in the inner aqueous phase is undesirable for successful encapsulation of the acid pH sensitive polypeptide.

33. The microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 comprising a blend of uncapped and capped polymer, wherein complete

solubilization of the copolymer leaves no residual polymer at the site of administration and occurs concurrently with the complete release of the entrapped agent.

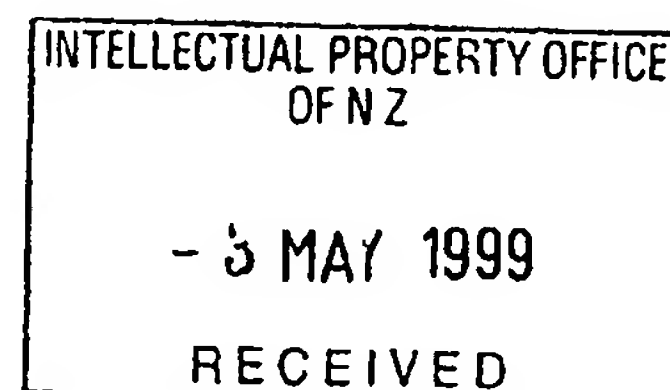
34. A process of using microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 for human administration via parenteral routes, such as intramuscular and subcutaneous.

35. A process of using microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 for human administration via topical route.

36. A process of using microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 for human administration via oral routes.

37. A process of using microcapsules of items 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 for human administration via nasal, transdermal, rectal, and vaginal routes.

Items 34 to 37 are claimed in New Zealand patent application No 335409 which has been divided from this application



Conservation of bioactivity of polypeptides

As the polymer degrades rapidly, there is a precipitous drop in pH accompanied by the release of soluble oligomers in the microenvironment which may affect the biological activity of acid pH-sensitive peptides/proteins. In such instances, biological activity can be maintained by the use of inorganic salts or buffering agents in the inner aqueous phase codissolved with the peptide.

The following unique advantages are characteristics of this invention:

1. Burst-free, prolonged, sustained release of polypeptides and other biocompatible and biodegradable microcapsules up to 100 days in an aqueous physiological environment without the use of additives in the core.

2. Release of active core programmable for variable durations over 1-100 days, by using a blend of uncapped and capped polymer of different molecular weights and copolymer ratio, and by manipulating the process parameters.

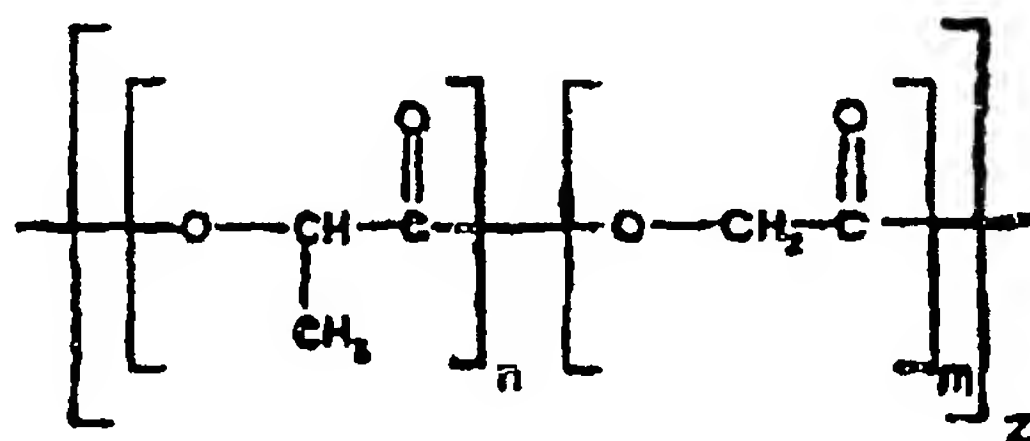
3. Complete release of the active core is concurrent with complete solubilization of the carrier polymer to innocuous components, such as lactic and glycolic acids, especially when using a 100/0 blend of uncapped/capped polymer. This is of tremendous significance, as most biodegradable polymers currently used for 1-30 day delivery, do not degrade completely at the end of the intended release duration, thereby causing serious concern

of regulatory authorities on the effects of residual polymer at the site of administration.

4. Ease of administration of the microcapsules in various dosage forms via several routes, such as parenteral (intramuscular and subcutaneous), oral, topical, nasal, vaginal, etc.

The hydrophilic homo-and co-polymers based on D,L-lactide and glycolide contains hydrophilic adjusted homo-and co-polymers with free carboxylic end groups, and is characterized by the formula:

Poly(D,L-lactide-co-glycolide) 50:50



Wherein  $Z = \text{Molecular Weight}/130$ ; for example  $Z=92$  for Mw 12,000 and 262 for Mw 34,000.

While the molar ratio of the lactide to glycolide may vary, it is most preferred that the lactide to glycolide copolymer ratio be 50:50.

Reference is now made to FIG. 1 which depicts a blood-drug concentration versus time graph that shows conventional drug administration using a series of dosages compared to an ideal controlled release system. Unfortunately, many drugs have a

therapeutic range, above which they are toxic and below which they are ineffective. Oscillating drug levels that are commonly observed following systemic administration causes alternating periods of ineffectiveness and toxicity. A sustained-release encapsulated biologically active agent or polypeptide preparation, ideally, will maintain the drug in the desired therapeutic range by means of a single dose, as depicted in the THERAPEUTIC RANGE in FIG.1 , where the ideal case for controlled release is shown.

10 In FIG. 2, there is shown a scanning electron micrograph of PLGA microspheres prepared using 50/50 uncapped polymer of Mw 8-12k dalton. The uncapped polymer has solid, smooth spherical surfaces, and is suited to provide a "burst free" release system.

Table I is a summarization of the microsphere process description for preparing a peptide system (Histatin peptide) having a controlled release over the course of from 1 to 100 days.

Release profiles can be modified by a judicious blend of uncapped and capped polymers either in separate microspheres or in the same microspheres. Release from microcapsule formulations 1 through 21 listed in Table 1, occur independently of each other and hence the cumulative release from blends of these formulations are additive. By blending several formulations of uncapped and end-capped microspheres, release curves of any desired duration can be tailored. In addition, based on the release characteristics of uncapped and end-capped polymers,

blending of the two forms in a single formulation comprising  
different ratios of uncapped to capped polymer, would  
significantly influence the polymer hydration and hence release  
of the active core thereby providing release curves of any  
5 desirable pattern. Manipulation of polymer hydration and  
degradation resulting in modulation of release of active core is  
achieved by the addition of uncapped polymer to end-capped  
polymer in amounts as low as 1% up to 100%.

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Table 1- Microcapsule compositions containing Histatin polypeptide

Composition #	Polymer Description			Theoretical peptide Core Load (%)	Internal Phase Ratio (w/o)	Emulsification Technique
	L/G Ratio & Type	Mol. Wt. (M w x 10 <sup>3</sup> )	Conc. in DCM (w/w)			
1.	50/50, U	12	38	5	1:20	A
2.	50/50, U	12	18.5	2	1:20	A
3.	50/50	34	10	5	1:20	A
4	50/50, U	12	38	5	1:4	A

5.	50/50	34	7	5	1 10	B
6.	50/50	34	10	5	1:10	B
7.	50/50	34	10	5	1:10	A
8.	75/25	12	10	5	1:10	B
9.	75/25	12	23 5	5	1 10	B
10.	50/50	12	10	5	1:10	B
11.	50/50	12	7	5	1:10	B
12.	50/50 ,U	12	10	5	1:10	B
13.	50/50 ,U	12	7	2.3	1:10	B



14.	50/50 ,U	12	10	5	1:10	B
15.	50/50 ,U	34	10	5	1:10	B
16.	50/50 ,U	12	10	5	1:10	B
17.	50/50 ,U	12	20	5	1:10	B
18.	50/50 ,U	12	40	5	1:10	B
19.	50/50 ,U	34	5	5	1:10	B
20.	50/50 ,U	34	10	5	1:10	B
21.	50/50 U	34	15	5	1:10	B

**Acronyms:**

L/G ratio: Copolymer composition of lactide/glycolide

DCM: Methylene Chloride

Mw: Molecular weight in daltons

A: w/o/w emulsification without an intermediate step for emulsion stabilization

B: w/o/w emulsification with an intermediate step for emulsion stabilization

U: Uncapped polymer

While referring to Table 1 in conjunction with FIG. 3, it can be seen that the cumulative Histatin release from PLGA microspheres from several batches prepared using 50/50 and 75/25 uncapped and end-capped, polymer modulates release between 1 to 100 days by varying the process parameters. 1-35 days by uncapped 50/50, 18-56 days by capped 50/50 and 56-100 days by capped 75/25.

In referring to FIG. 4, a view is provided through a scanning electron micrograph of PLGA microspheres designed for a one to two month release system prepared using end-capped polymer of Mw 30-40k daltons.

FIG. 5 depicts the cumulative Histatin release from PLGA microspheres, in which the release profiles are from several batches prepared using 50/50, uncapped and capped polymer, and varying the process parameters to modulate release between 28 to 60 days.

Figure 6 represents cumulative Histatin release from PLGA microspheres --- these combined release profiles are from several batches prepared using 50/50 uncapped and capped polymer, and varying the process parameters to modulate release between 1-60 days.

In the context of the invention, a biologically active agent is any water-soluble antibiotics, antitumor agents, antipyretics, analgesics, anti-inflammatory agents, antitussives, expectorants, sedatives, muscle relaxants, anti epileptics, antiulcer agents,

anti-depressants, anti-allergic drugs, cardiotonics,  
antiarrhythmics drugs, vasodilators, antihypertensives,  
diuretics, anticoagulants, hormone drugs, anti-narcotics, etc.

In general, "burst free" sustained release delivery of  
5 biologically active agents from PLGA microspheres is accomplished  
in the context of this invention using of 90/10 to 40/60 molar  
ratios, and ratios of uncapped polymer to end-capped polymer of  
100/0 to 1/99.

In general, the approaches for designing the biologically  
10 active agents encapsulated in the uncapped and combination  
uncapped/end-capped PLGA microspheres and characteristics of  
these encapsulants are briefly set forth below as follows:

1. Providing PLGA microspheres of surface morphologies using  
15 50/50 uncapped and capped polymers of Mw - 8-40K daltons as shown  
in Figs. 2 and 4.

2. Providing in vitro release of a polypeptide, Histatin  
from PLGA microspheres, as shown in Figs. 3 and 5, using uncapped  
20 and capped polymer of Mw - 8-40K daltons and molar ratios such as  
50/50 and 75/25.

For example, design of a 1-12 week bioactive compound  
release system is achieved using PLGA with the following  
25 specifications:

1. Polymer molecular weight:  
- about 2-60K daltons
2. Copolymer molar ratio (L/G):  
- 90/10 to 40/60
- 30 3. Polymer end groups:  
- uncapped and /or end-capped

and combining judiciously within the following parameters:

- 35 4. Polymer concentration  
- from 5 to 50%
5. Inner aqueous to oil phase ratio:  
- 1:5 to 1:20 (v/v)
- 40 6. Peptide loads:  
- from 2 to about 40% (w/w polymer)

and by using the unique aqueous emulsification method described in the invention.

5 The uniqueness and novelty of invention may generally be summarized in a brief way as follows:

1. Use of uncapped poly(lactide/glycolide) to achieve burst-free, continuous, sustained, programmable release of biologically active agents over 1-100 days.

10 2. Use of a unique aqueous emulsification system to achieve superior microsphere characteristics such as uniform sphere morphology and narrow size distribution.

15 3. Burst-free, prolonged, sustained release of polypeptides and other biologically active agents from biocompatible and biodegradable microcapsules up to 100 days in an aqueous physiological environment without the use of additives in the inner core.

20 4. Release of active core programmable for variable durations over 1-100 days by using a blend of uncapped and capped polymer for different molecular weights and copolymer ratios and manipulating the process parameters.

25 5. Complete release of the active core concurrent with complete solubilization of carrier polymer to innocuous components such as lactic and glycolic acids, especially when using a 100/0 blend of uncapped/capped polymer. This is of tremendous significance as most biodegradable polymers currently in use for 1-30 day delivery, do not degrade completely at the end of the intended release duration causing serious concern for regulatory authorities on the effects of residual polymer at the site of administration.

30 6. Ease of administration of the microcapsules in various dosages, forms via several routes such as parenteral (intramuscular and subcutaneous), oral, topical, nasal, vaginal, etc.

35 The following examples are illustrative of, but not limitations upon the microcapsule compositions pertaining to this invention.

#### 45 Example 1

50 Polylactide/glycolide (PLGA) microcapsules are prepared by a unique aqueous emulsification technique which has been developed for use with the uncapped polymer to provide superior sphere morphology, sphere integrity and narrow size distribution (See Figures 1a and 1b). This is accomplished by dissolving the polymer in a chlorinated hydrocarbon solvent such as methylene chloride and dissolving the biologically active agent in water. A

w/o emulsion is then formed by mixing the solutions of polymer and the active agent by sonication, followed by emulsion stabilization in a solvent - saturated aqueous solution containing polyvinyl alcohol. A ternary emulsion is then formed by emulsifying the w/o emulsion in an external, pre-cooled aqueous phase containing polyvinyl alcohol (0.25 - 1% w/v). Microcapsules are hardened upon removal of solvent by evaporation, rinsed to remove any residual emulsifier, and then lyophilized.

Table 1 lists the microcapsule compositions, Nos. 1-21 thus prepared, consisting of a biologically active polypeptide, Histatin (composed of 12 amino acids and a molecular weight of 1563) and blends of uncapped and capped polymer of ratios 100/0 to 1/99, and having a lactide/glycolide ratio of 90/10 to 40/60, and a molecular weight range between 2000 to 60,000 daltons.

#### Example 2

Microcapsule compositions are prepared as described in Example 1 wherein the copolymer L/G ratio is 48/52 to 52/48, and the ratio of uncapped/capped polymer is 100/0. The active core is Histatin (Mw 1563), the polymer molecular weight is < 15,000 and the polymer concentrations vary from 7% to - 40% w/w. Compositions 1,2,4 12-14 and 16-18 are listed in Table 1.

Release profiles of the active core from the compositions in an aqueous physiological environment, such as phosphate-buffered saline, pH 7.0 maintained at  $37 \pm 1^\circ\text{C}$  are plotted as cumulative percentage release versus time, and presented in Figure 2.

Burst-free, variable release from 1-35 days is achieved by varying the polymer concentration from 7 to - 40% w/w in the oil phase.

#### Example 3

Microcapsule compositions are prepared as described in Example 2, wherein the aqueous /oil ratio is varied from 1/4 to 1/20 (v/v). Compositions 1,2,4 and 12 are listed in Table 1.

Release profiles of the active core from the compositions in an aqueous physiological environment described in Example 2 are plotted as cumulative percentage release versus time, and presented in Figure 2.

Burst-free, continuous release from 1-35 days, with different onset and completion times are achieved by selecting

different w/o ratios in the inner core.

Example 4

5        Microcapsule compositions are prepared as described in Example 2, wherein the polymer molecular weight is 28,000-40,000 and polymer concentrations vary from 5% to ~ 15% w/w. Compositions 19-21 are listed in Table 1.

10       Release profiles of the active core from the compositions in an aqueous physiological environment are described in Example 2 are plotted as cumulative percentage release versus time and presented in Figure 3.

15       Burst-free, variable release from 18-40 days is achieved by varying the polymer concentration.

Example 5

20       Microcapsule compositions are prepared as described in Example 2, wherein the ratio of uncapped/capped polymer is 1/99 and polymer concentrations vary between 5% to ~ 12% w/w. Compositions 10 and 11 are listed in Table 1.

25       Release profiles of the active core from the compositions in an aqueous physiological environment are described in Example 2, and plotted as cumulative percentage release versus time and presented in Figure 2.

30       Burst-free, variable release from 28-70 days is achieved by varying the polymer concentration in the oil phase

Example 6

35       Microcapsule compositions are prepared as described in Example 5, wherein polymer molecular weight is 28,000-40,000 and polymer concentrations vary between 5% to ~ 12% w/w. Compositions 5 and 6 are listed in Table 1.

40       Release profiles of the active core from the compositions in an aqueous physiological environment are described in Example 2 and are plotted as cumulative percentage release versus time, and presented in Figure 3.

45       Burst-free, variable release from 28-70 days is achieved by varying the polymer concentration.

Example 7

50       Microcapsule compositions are prepared as described in Example 6, wherein the aqueous/oil ratio varies between 1/5 to



1/25 (v/v). Compositions 3 and 7 are listed in Table 1.

5 Release profiles of the active core from the compositions in an aqueous physiological environment are described in Example 2, and plotted as cumulative percentage release versus time, and presented in Figure 3.

10 Burst-free, variable release from 28-70 days is achieved by varying the aqueous/oil ratios.

#### Example 8

15 Microcapsule compositions are prepared as described in Example 5, wherein the copolymer ratio is 75/25 and polymer concentrations vary between 5% to ~ 25% w/w. Compositions 8 and 9 are listed in Table 1.

20 Release profiles of the active core from the compositions in an aqueous physiological environment are described in Example 2, and are plotted as cumulative percentage release versus time, and presented in Figure 2.

25 Burst-free, variable release from 56-90 days is achieved by varying the polymer concentration in the oil phase.

#### Example 9

30 Microcapsule compositions are described in Example 2, wherein the active core is luteinizing hormone releasing hormone (LHRH, a decapeptide of molecular weight 1182) and the polymer concentration is ~40% w/w. Release profiles of the active core from the composition in an aqueous physiological environment is described in Example 2, and is plotted as cumulative percentage release versus time, and presented in Figure 4.

35 Burst-free, continuous and complete release is achieved within 35 days, similar to Histatin acetate.

40

#### Example 10

45 Microcapsule compositions are prepared as described in Example 2, wherein an additive such as sodium salt (carbonate or bicarbonate) is added to the inner aqueous phase at concentrations of 1-10% w/w to maintain the biological activity of the released polypeptide.

50 Burst-free, variable release from 1-28 days is achieved similar to Examples 2 & 3, and the released polypeptide is biologically active until 30 days, due to the presence of the sodium salt.



Example 11

Microcapsule compositions are prepared as described in Example 2, wherein an additive such as a nonionic surfactant, polyoxyethylene/polyoxypropylene block copolymer (Pluronic F68 and F127) is added to either the inner oil or the aqueous phase at concentrations from 10-100% w/w, to maintain the biological activity of the released polypeptide.

Burst-free, continuous release from 1-35 days is achieved similar to Examples 2 & 3, and the released polypeptide is bioactive due to the presence of the surfactant.

Example 12

Cumulative histatin release from the microcapsule compositions described in Examples 1 through 11 and release profiles plotted in Figures 2 and 3 show the burst-free, programmable peptide release for variable duration from 1-100 days. Virtually any pattern of cumulative release is achievable over a 100 day duration by a judicious blending of several compositions, as shown in these figures.

## WE CLAIM

1. A controlled release microcapsule pharmaceutical  
formulation for burst-free, sustained, programmable release of a  
5 biologically active agent over a duration from 1-100 days,  
comprising an active agent and a blend of uncapped and end-capped  
biodegradable poly(lactide/glycolide).

2. The pharmaceutical formulation of claim 1, wherein the  
biodegradable poly(lactide/glycolide) is a blend of uncapped and  
10 capped forms, in ratios ranging from 100/0 to 1/99.

3. The microcapsules of claims 1 or 2 wherein the copolymer  
(lactide to glycolide L/G) ratio for uncapped and endcapped  
polymer is 52/48 to 48/52.

4. The microcapsules of claims 1 or 2 wherein the copolymer  
15 L/G ratio for uncapped and end-capped polymer is 90/10 to 40/60.

5. The microcapsules of claim 3 wherein the molecular weight  
of the copolymer is between 2,000-60,000 daltons.

6. The microcapsules of claim 1 wherein the biologically  
active agent is a peptide or polypeptide.

20 7. The microcapsules of claim 6, wherein said polypeptide is  
histatin consisting of 12 amino acids and having a molecular  
weight of 1563.

8. The microcapsules of claim 7 characterized by the  
capacity to completely release histatin in an aqueous  
25 physiological environment from 1-35 days with a 100/0 blend of  
uncapped and end-capped poly(lactide/glycolide) having a L/G

ratio of 48/52 to 52/48, and a molecular weight <15,000.

9. The microcapsules of claim 7 characterized by the capacity to completely release histatin in an aqueous physiological environment from 18-40 days with a 100/0 blend of uncapped and end-capped poly(lactide/glycolide) having a L/G ratio of 48/52 to 52/48 and a molecular weight range of 28,000-40,000.

10. The microcapsules of claim 7 characterized by the capacity to release up to 90% of the histatin in an aqueous physiological environment from 28-70 days with a 100/0 blend of uncapped and end-capped poly(lactide/glycolide) having a L/G ratio of 48/52 to 52/48 and a molecular weight range of 10,000-40,000 daltons

11. The microcapsules of claim 7 characterized by the capacity to release up to 80% of histatin in an aqueous physiological environment from 56-100 days with a 100/0 blend of uncapped and end-capped poly(lactide/glycolide) having a L/G ratio of 75/25 and a molecular weight of < 15,000 daltons

12. The microcapsules of claim 6 wherein the polypeptide is histatin having analogs of histatin with chain lengths of from 11-24 amino acids of molecular weights from 1,500-3,000 daltons and characterized by the following structures:

1. D S H A K R H H G Y K R K F H E K H H S H R G Y

2. K R H H G Y K R K F H E K H H S H R G Y R

3. K R H H G Y K R K F H E K H H S H R

4. R K F H E K H H S H R G Y R

5.           A K R H H G Y K R K F H

6.           \* A K R H H G Y K R K F H

7.           K R H H G Y K R K F

\* D-amino acid

3 2 5 5 9 1

5           13. The microcapsules of claim 6 wherein the biologically active agent is a polypeptide Luteinizing hormone releasing hormone (LHRH) that is a decapeptide of molecular weight 1182 in its acetate form, and having the structure:

p- E H W S Y G L R P G

10

14. The microcapsule of claim 6 having a molecular weight of from 1,000 to 250,000 daltons.

15          15          The microcapsules of claims 6 or 7 or 8 or 9 or 10 or 11 or 13 or 14 wherein release profiles of variable rates and durations are achieved by blending uncapped and capped biodegradable poly(lactide/glycolide) polymer in the microspheres as a cocktail in variable amounts

16          16          The microcapsules of claims 6 or 7 or 8 or 9 or 10 or 11 or 13 or 14 wherein release of profiles of variable rates and duration are achieved by blending uncapped and capped polymer in different ratios within the same microspheres

20

17          17          The microcapsules of claim 6 wherein the polypeptide is any of the vaccine agents against enterotoxigenic E. coli (ETEC) such as CFA/I, CFA/II, CS1, CS3, CS6 and CS17 and other ETEC-related enterotoxins.

25

18. The microcapsules of claims 6 or 7 or 8 or 9 or 10 or 11 or 13 or 14 wherein the entrapped polypeptide consists of peptide antigens of molecular weight range of about 800-5000 daltons for immunization against enterotoxigenic E. coli (ETEC).

5 19. The microcapsules of claims 1 or 2 or 3 or 4 or 5 wherein said biologically active agents are selected from the group consisting of water-soluble hormone drugs, antibiotics, antitumor agents, anti inflammatory agents, antipyretics, analgesics, antitussives, expectorants, sedatives, muscle  
10 relaxants, antiepileptics, antiulcer agents, antidepressants, antiallergic drugs, cardiotonics, antiarrhythmic drugs, vasodilators, antihypertensives, diuretics, anticoagulants, and antinarcotics, in the molecular weight range of 100-100,000 daltons.

15 20. The microcapsules of claims 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 wherein said biodegradable poly(lactide/glycolide) is in an oil phase, and is present in about 1-50% (w/w).

21. The microcapsules of claims 1 or 2 or 3 or 4 or 5 or 6 or 8 or 9 or 10 or 11 or 13 or 14 wherein concentration of the  
20 active agent is in the range of 0.1 to about 50% (w/w).

22. The microcapsules of claims 1 or 2 or 3 or 4 or 5 or 6 or 8 or 9 or 10 or 11 or 13 or 14 wherein a ratio of the inner aqueous to oil phases is about 1/4 to 1/40(v/v).

23. A process for preparing controlled release microcapsule  
25 formulations characterized by burst-free, sustained, programmable release of biologically active agents comprising: Dissolving

biodegradable poly (lactide/glycolide), in uncapped form in methylene chloride, and dissolving a biologically active agent or active core in water; adding the aqueous layer to the polymer solution and emulsifying to provide an inner water-in-oil (w/o) emulsion; stabilizing the w/o emulsion in a solvent-saturated aqueous phase containing a oil-in-water (o/w) emulsifier; adding said w/o emulsion to an external aqueous layer containing oil-in-water emulsifier to form a ternary emulsion; and stirring the resulting water-in-oil-in-water (w/o/w) emulsion for sufficient time to remove said solvent, and rinsing hardened microcapsules with water and lyophilizing said hardened microcapsules.

24. A process for preparing controlled release microcapsule formulations characterized by burst-free, sustained, programmable release of biologically active agents comprising:

dissolving biodegradable poly(lactide/glycolide) in uncapped form in methylene chloride, and dissolving a biologically active agent or active core in water; adding the aqueous layer to the polymer solution and emulsifying to provide an inner water-in-oil emulsion; stabilizing the w/o emulsion in a solvent-saturated aqueous phase containing a oil-in-water (o/w) emulsifier; adding said w/o emulsion to an external aqueous layer containing oil-in-water emulsifier to form a ternary emulsion; and stirring a resulting water-in-oil-water (w/o/w) emulsion for sufficient time to remove said solvent; and rinsing hardened microcapsules with water; and lyophilizing said hardened microcapsules.

25. The process of claims 23 or 24 wherein a solvent-saturated external aqueous phase is added to emulsify the inner w/o emulsion prior to addition of the external aqueous layer, to provide microcapsules of narrow size distribution range between  
5 0.05-500 $\mu$ m.

26. The process of claims 23 or 24, wherein a low temperature of about 0-4°C is provided during preparation of the inner w/o emulsion, and a low temperature of about 4-20°C is provided during preparation of the w/o/w emulsion to provide a  
10 stable emulsion and high encapsulation efficiency.

27. The process of claim 23 wherein a 100/0 blend of uncapped and end-capped polymer is used to provide release of the active core in a continuous and sustained manner without a lag phase.

15 28. The microcapsules of claim 6, wherein, when the entrapped polypeptide is active at a low pH, such as LHRH, adrenocorticotrophic hormone, epidermal growth factor, calcitonin released polypeptide is bioactive.

20 29. The microcapsules of claims 6 or 7 or 8 or 9 or 10 or 11, wherein, when entrapped peptide such as histatin is inactive at a low pH, a pH-stabilizing agent of inorganic salts are added to the inner aqueous phase to maintain biological activity of the released peptide.

25 30. The microcapsules of claims 6 or 7 or 8 or 9 or 10 or 11 wherein, when entrapped polypeptide such as histatin is inactive at a low pH, a non-ionic surfactant such as polyoxyethylene

3 2 5 5 0 1 5

sorbitan fatty acid esters (Tween 80, Tween 60 and Tween 20) and polyoxyethylene - polyoxypropylene block copolymers (Pluronic) is added to the inner aqueous phase to maintain biological activity of the released polypeptide.

5           31. The microcapsules of claim 29, wherein placebo spheres loaded with the pH-stabilizing agents are coadministered with polypeptide-loaded spheres to maintain the solution pH around the microcapsules and preserve the biological activity of the released peptide in instances where the addition of pH-

10           stabilizing agents in the inner aqueous phase is undesirable for the successful encapsulation of the acid pH sensitive polypeptide.

          32. The microcapsules of claim 30 wherein placebo spheres loaded with non-ionic surfactant are coadministered with

15           polypeptide-loaded spheres to maintain biological activity of the released peptide where the addition of non-ionic surfactants in the inner aqueous phase is undesirable for successful encapsulation of the acid pH sensitive polypeptide.

          33. The microcapsules of claims 1 or 2 or 3 or 4 or 5 or 6

20           or 8 or 9 or 10 or 11 or 13 or 14 comprising a blend of uncapped and capped polymer, wherein complete solubilization of the copolymer leaves no residual polymer at the site of administration and occurs concurrently with the complete release of the entrapped agent.

25           34     A microcapsule pharmaceutical formulation of claim 1 substantially as herein described with reference to any one of Examples 1 to 11.

          35     A process for preparing a microcapsule pharmaceutical formulation of claim 1 substantially as herein described with reference to any one of Examples 1 to

11

**END OF CLAIMS**



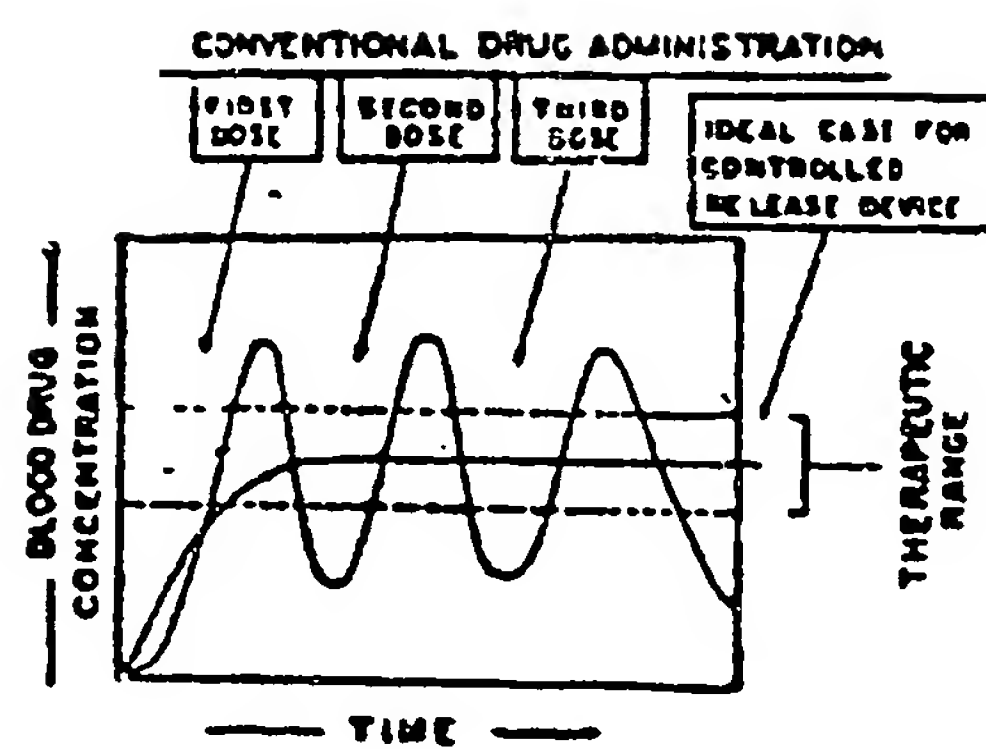


Fig. 1

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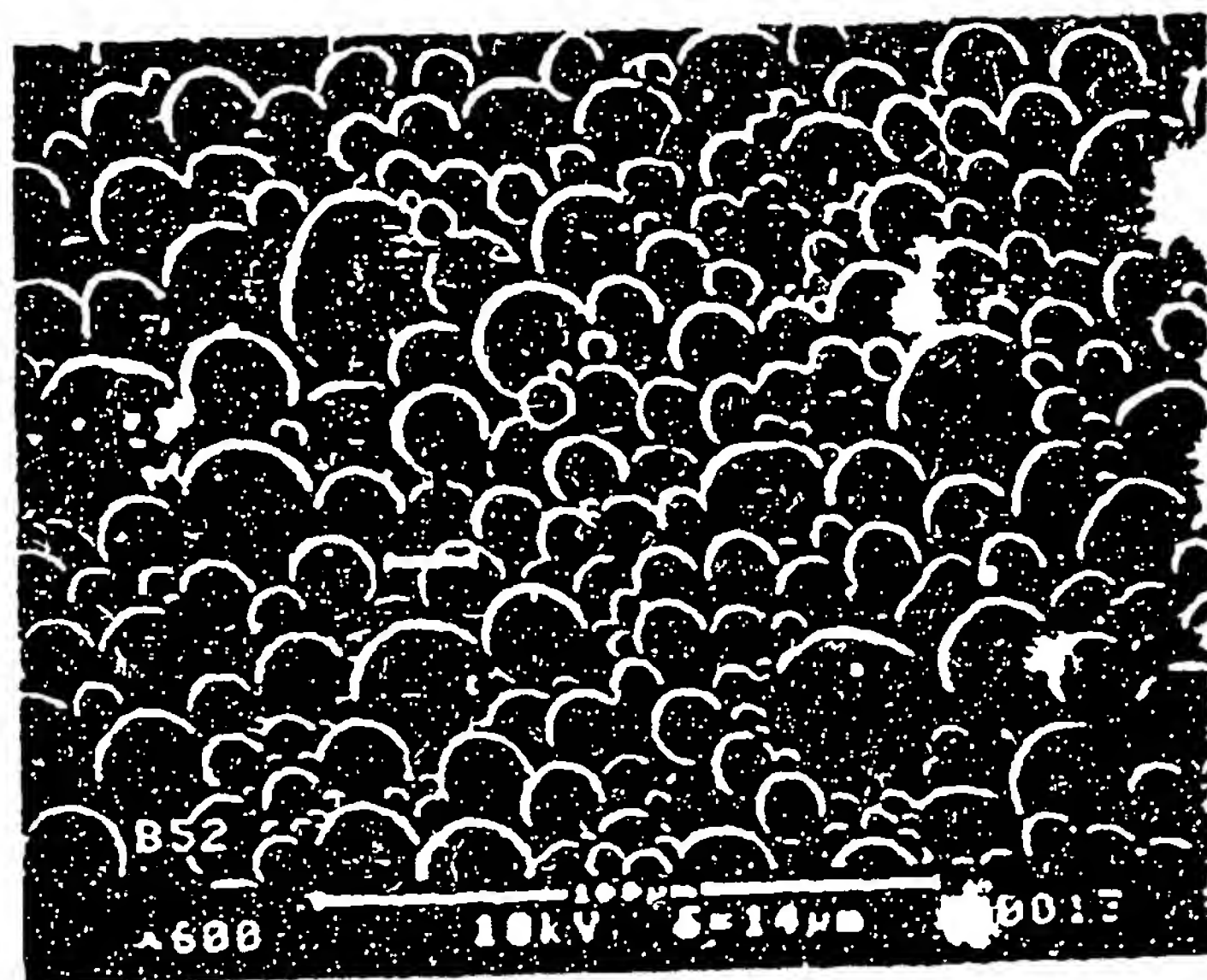


Fig. 2

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Figure 2a

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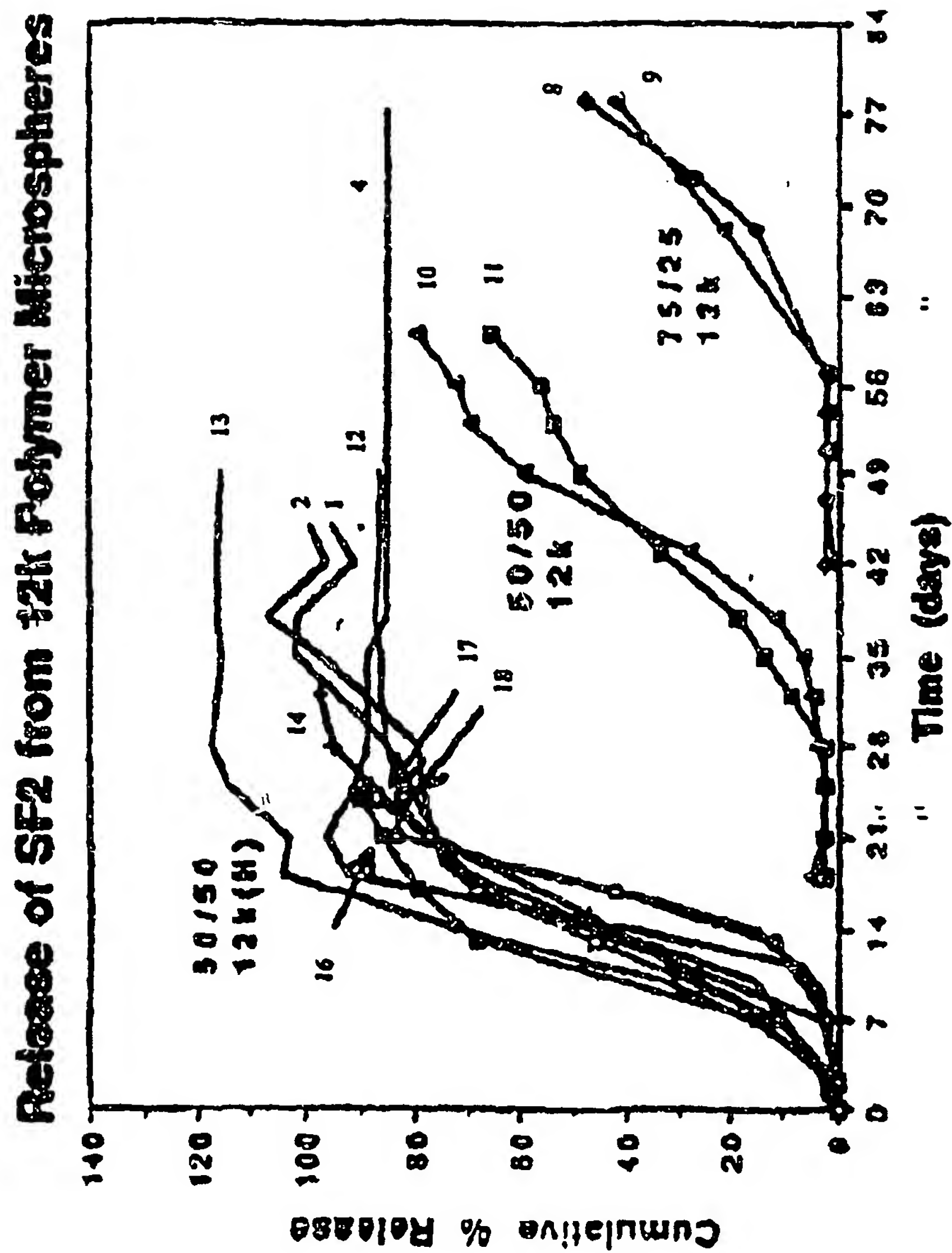


Fig. 3

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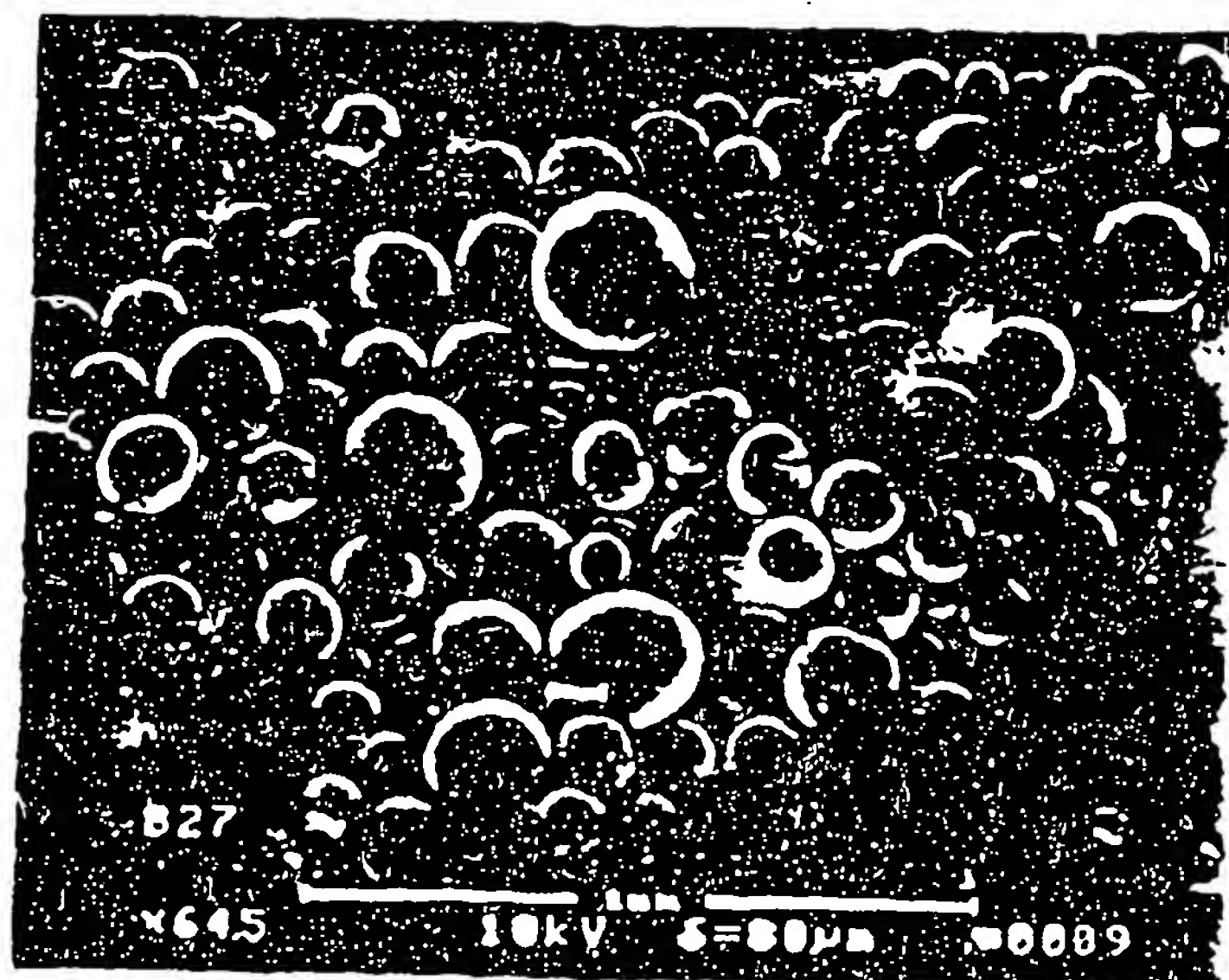


Fig. 4

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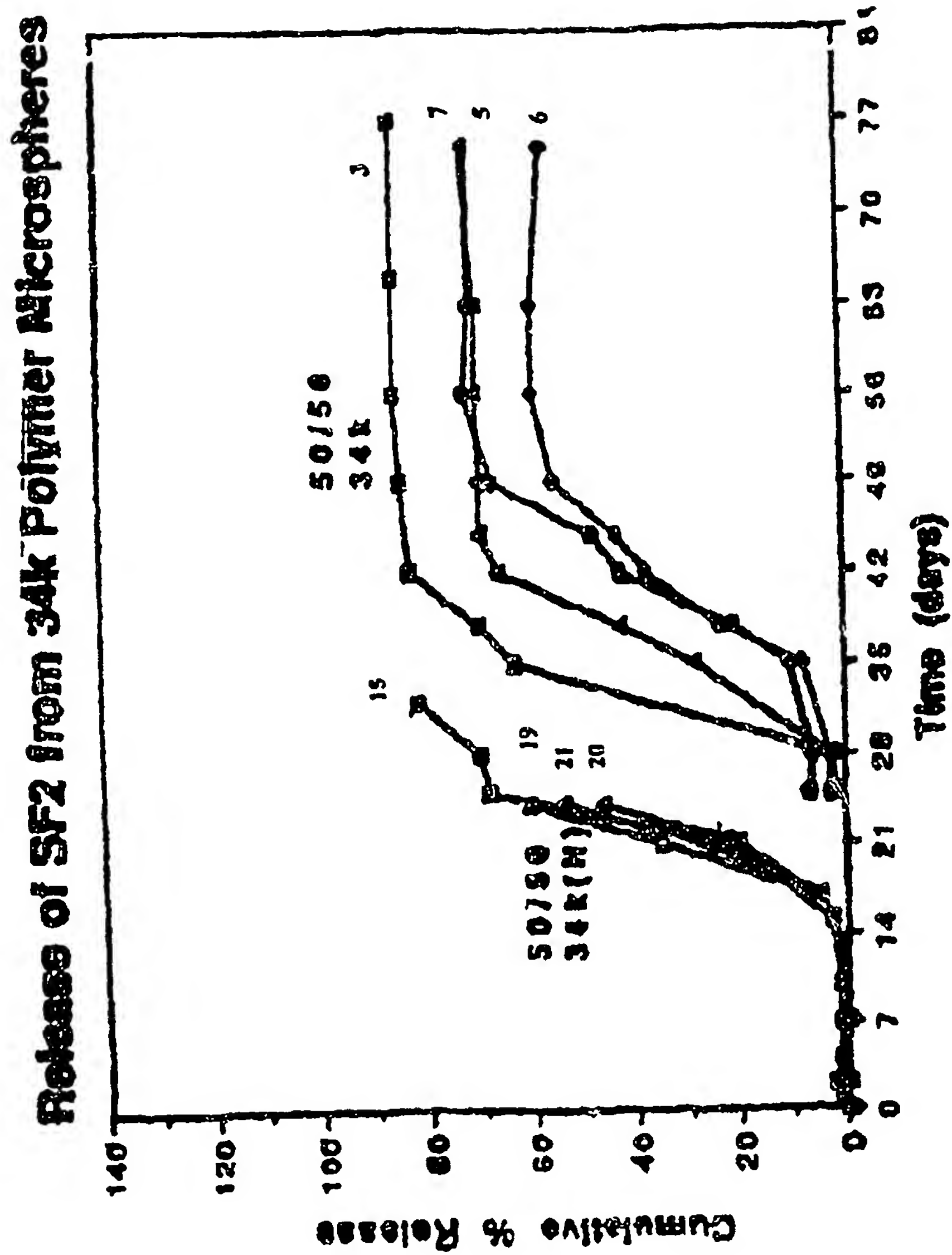


Fig. 5

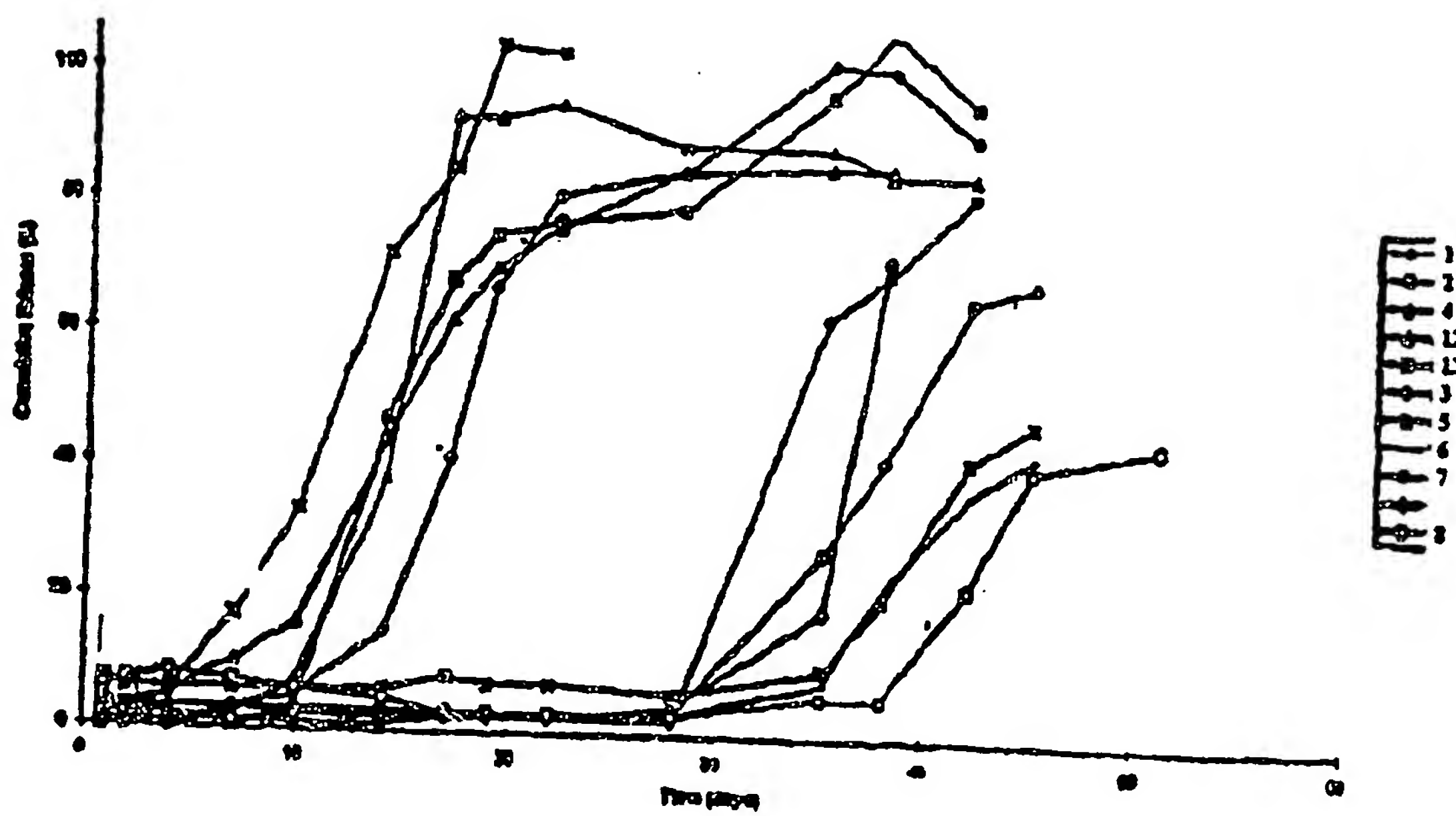


Fig. 6

Sheet 1 Chart 1

